STUDIES OF THE CEREBROSPINAL FLUID IN ACUTE ANTERIOR POLIOMYELITIS.*

WITH SPECIAL REFERENCE TO THE COLLOIDAL GOLD, POTASSIUM PERMANGANATE REDUCTION, WEIL-KAFKA HEMOLYSIN, AND SAPONIN REACTIONS.†

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The results of the examination of cerebrospinal fluids in acute anterior poliomyelitis summarized in this report are compiled from the routine examinations and special studies‡ of cerebrospinal fluids from patients admitted to the Philadelphia Hospital for Contagious Diseases¶ during the epidemic of the summer of 1916, in an effort to contribute to our knowledge of the changes occurring in the spinal fluid in this disease and the value of its examination as a practical means of diagnosis.

Careful histological studies of the spinal meninges and cords of early experimental and human poliomyelitis infections have shown that the primary reaction of the nervous system is the production of an acute meningitis characterized by hyperemia, edema, and perivascular round-cell infiltration in the pia mater, with subsequent extension into the substance of the cord and involvement of the anterior horn cells. The spinal fluid may be altered by the passage into it of cells from the perivascular and interstitial exudate in the meninges and of serum constituents derived from the hyperemic bloodvessels.

The investigations of Gay and Lucas,[1] and Lucas,[2] confined principally to a study of the cells in the spinal fluids of monkeys experimentally infected and of 11 cases in the preparalytic stage, have shown that the total cell count of the fluid is increased considerably above the normal, the majority of the cells being of the mono-

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¶ These patients were under the care of Dr. S. S. Woody, chief resident physician, and Dr. Theodore Le Boutillier, Dr. Theodore Weisenberg, Dr. William Drayton and Dr. Maurice Ostheimer of the visiting staff of the hospital.

nuclear variety. Flexner and Lewis[3] in a study of the spinal fluid from a monkey after inoculation with the virus found a considerable increase in the number of cells twenty-four hours after inoculation, and particularly a small cell slightly larger than a lymphocyte showing a polyform nucleus. Sophian[4] reported an increase of globulin in the spinal fluid of cases in the early stages. In a thorough study of 233 fluids from 69 cases, Peabody, Draper and Dochez[5] found an increased cell count, with a low or normal globulin content, in fluids taken during the early days of the disease and before the onset of paralysis; at this time the polymorphonuclear cells amounted to 90 per cent. of the total, although most fluids showed almost exclusively lymphocytes and large mononuclear cells. According to these investigators analogous changes may be found in the spinal fluid of abortive cases; all fluids examined reduced Fehling's solution. They conclude that "the examination of the cerebrospinal fluid in acute poliomyelitis, while giving, as far as is yet known, no specific diagnostic criteria, is of the utmost value as an aid to diagnosis both in preparalytic and in abortive cases." More recent reports of similar findings have been made by Neal and Du Bois[6] and Abramson.[7]

During the recent epidemic of poliomyelitis in Philadelphia we have examined 868 specimens of cerebrospinal fluid from cases of epidemic poliomyelitis admitted to the Philadelphia Hospital for Contagious Diseases and in private practice. In a smaller number of these the histories and clinical studies were sufficiently accurate for purposes of analysis and correlation with the results of laboratory examinations, and the major portion of this laboratory report is based upon these.

1. Physical Properties of the Spinal Fluid in Acute Anterior Poliomyelitis. The majority of the spinal fluids examined by us were water-clear or showed but a faint opalescence when viewed against a black background. Of 316 fluids from cases early in the stage of paralysis, but 5, or from 1 to 2 per cent. of blood-free fluids, were distinctly turbid. Of the fluids of 6 cases which we had the opportunity of examining in the preparalytic stage 2 showed a faint opalescence and 4 were clear. The 5 distinctly turbid fluids were from cases on the fourth, fourth, fifth, sixth, and sixth days after the onset of paralysis, and showed total cell counts of more than 200 cells per cubic millimeter, many of which were polymorphonuclears.

A small fibrin coagulum was noticed in about 10 per cent. of the spinal fluids a few hours after they had been collected and standing in test-tubes. We have not observed xanthochromia or excessive fibrin formation in any specimen.

Many specimens contained small amounts of blood, and while the presence of blood may have been due in certain cases to extravasation from the hyperemia vessels of the pia, we are of the opinion that in the majority of these fluids, blood gained access by reason of the puncture of vessel with the needle, as only the first flow of fluid contained blood.

2. CYTOLOGY OF THE SPINAL FLUID IN ACUTE ANTERIOR POLIOMYELITIS.* The results of total cell counts with 787 fluids from cases of epidemic poliomyelitis in all stages of the disease are shown in Table I.

TABLE I.—SUMMARY OF RESULTS OF TOTAL CELL COUNTS OF SPINAL FLUIDS IN ACUTE ANTERIOR POLIOMYELITIS.

Cells per cubic millimeter of fluid.				
0 to 12.	13 to 50.	51 to 100.	101 to 500.	Over 500.
178	328	156	95	30

If the upper limit of the normal number of cells per cubic millimeter of cerebrospinal fluid is placed at 12, of these fluids 609, or 77 per cent., showed an increase of cells. In the majority of these the increase of cells was moderate, as 80 per cent. of those fluids containing an increased number of cells the counts were not above 100 cells per cubic millimeter of fluid.

The results of total cell counts in 319 fluids from patients in various stages of epidemic poliomyelitis are classified in Table II according to the day *after the onset of paralysis*. This method of classification was necessary because many cases were not diagnosed or sent to the hospital until paralysis was present, and histories of the preparalytic stage were inaccurate. The cell counts of five cases from private practice in the preparalytic stage are included.

TABLE II.—RESULTS OF TOTAL CELL COUNTS IN VARIOUS STAGES OF POLIOMYELITIS AFTER THE ONSET OF PARALYSIS (319 FLUIDS).

Day of disease.	Total exam.	Cells per cubic millimeter.					Percentage showing increase.
		0-12.	13-50.	51-100.	101-500.	Over 500.	
Preparalytic	5	..	3	2	..	..	100
1 to 3 days	125	19	52	26	24	4	84
4 to 7 "	99	17	51	19	9	3	82
8 to 14 "	55	9	32	8	6	..	83
15 to 21 "	21	8	11	1	1	..	62
22 to 28 "	10	7	2	1	..	..	28
29 to 35 "	4	3	1	..	..	..	25

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* In making total cell counts the ordinary leukocyte mixing pipet and the Fuchs-Rosenthal chamber were employed. The diluting fluid which stained the cells and hemolyzed any erythrocytes present was prepared as follows: glacial acetic acid, 1 c.c.; distilled water, 99 c.c.; methyl violet, crystals, 0.1 gm. This fluid was filtered before use. *Counts were made as soon as possible after spinal puncture and only of those fluids showing no macroscopic evidences of blood.* The diluting fluid was drawn into the pipet to the mark 1, the spinal fluid to 11, and the contents mixed by shaking for one minute. After loading the chamber and allowing the cells to settle for five to ten minutes all cells in the ruled-off space were counted and the number multiplied by 11 and divided by 32, which gave the total number of cells per cubic millimeter of fluid.

As shown in Table II an increase of cells was noted in all of the cases examined in the preparalytic stage, and this increase of cells was found in about 95 per cent. of cases to persist for at least three weeks after the onset of paralysis, when the total number of cells gradually approached normal numbers.

The results of differential cell counts with 87 fluids from patients in various stages of the disease after the onset of paralysis are shown in Table III.

TABLE III.—RESULTS OF DIFFERENTIAL CELL COUNTS OF SPINAL FLUIDS IN ACUTE ANTERIOR POLIOMYELITIS AFTER THE ONSET OF PARALYSIS (87 FLUIDS).

Variety of cell.	Under 25 per cent. Cases.	Between 25 to 50 per cent. Cases.	Between 50 to 75 per cent. Cases.	Between 75 to 100 per cent. Cases.
Small lymphocyte .	3	3	39	42
Large mononuclears .	85	2		
Polymorphonuclears .	77	7	1	2
Endothelial . . .	all under 10 per cent.			

As shown in Table III the small lymphocyte variety of cell predominated in the majority of fluids. In over 96 per cent. of these fluids this cell constituted 25 per cent. or more of the cells present.

Cells of the type of large mononuclear leukocytes, while occasionally found, were seldom present in large numbers.

Polymorphonuclear leukocytes predominated in less than 1 per cent. of the fluids examined (3 cases) and in over 88 per cent. constituted less than 25 per cent. of the cells present.

As previously stated the majority of these fluids were from patients in whom paralysis had occurred, whereas the predominance of polymorphonuclear cells is more likely in the preparalytic stage of the disease.

3. PROTEIN CONTENT OF THE SPINAL FLUID IN ACUTE ANTERIOR POLIOMYELITIS. The routine test for the globulin-albumin content of these spinal fluids was the butyric acid test of Noguchi. Of 868 fluids from patients in various stages of the disease this technic yielded positive reactions with 32 per cent. (Table IV). A comparative study of several similar qualitative methods have shown wide variations in the delicacy of different methods as shown in Table IV.

TABLE IV.—SUMMARY OF RESULTS OF PROTEIN TESTS WITH DIFFERENT METHODS (868 FLUIDS).

Method.	Total exam.	Results. +	Results. –	Percentage positive.
Nonne-Apelt . . .	79	11	68	14
Noguchi	868	280	588	32
Pandy	79	24	55	30
Gordon	79	30	49	37
Kaplan	79	33	46	42

The oldest of these clinical laboratory methods for globulin-albumin in spinal fluid (Nonne-Apelt)* proved the least delicate, and the method of Kaplan, the most delicate. Gordon's method proved quite simple and delicate, while our results with the Pandy test were closely parallel with those of Noguchi's test. The main objections to the latter test and that of Kaplan is the employment of the ill-smelling dilution of butyric acid.

The results of globulin-albumin tests with spinal fluids from patients in various stages of poliomyelitis after the onset of paralysis and employing Noguchi's method is shown in Table V.

TABLE V.—RESULTS OF PROTEIN TESTS (NOGUCHI METHOD) IN VARIOUS STAGES OF ACUTE ANTERIOR POLIOMYELITIS (270 FLUIDS).

Day of disease.	Total exam.	Results. +	Results. –	Percentage positive.
Preparalytic . . .	6	1	5	16
1 to 3 days . .	113	33	80	29
4 to 7 " . .	72	30	42	41
8 to 14 " . .	43	23	20	53
15 to 21 " . .	18	10	8	55
22 to 28 " . .	11	3	8	27
29 to 35 " . .	7	1	6	14

Of the fluids of 6 cases examined in the preparalytic stage but 1 yielded a positive reaction; the percentage of positive reactions then became gradually higher to the third week after the onset of paralysis, when a rapid decrease in positive reactions became apparent. As previously stated, however, and as shown in Chart 1, the cells are increased in the preparalytic and early stages of the disease, the number dropping with the globulin content after the third week following the onset of paralysis.

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* *Method of Nonne-Apelt.*—This test was conducted by mixing in a small test-tube 1 c.c. of spinal fluid and 1 c.c. of a saturated solution of ammonium sulphate in water; after standing three minutes this tube was compared with a second tube containing cerebrospinal fluid only and an increase of protein was indicated by opalescence, turbidity, and visible precipitation.

Method of Noguchi.—This test was conducted by mixing in a small test-tube 0.2 c.c. of spinal fluid and 1 c.c. of a 10 per cent. solution of butyric acid in physiological salt solution followed by heating to the boiling-point; 0.2 c.c. of normal sodium hydrate was added followed by heating to boiling for a few seconds. Positive reactions were read when marked opalescence or definite floccule formation occurred after standing for an hour. Faint opalescence was regarded as negative.

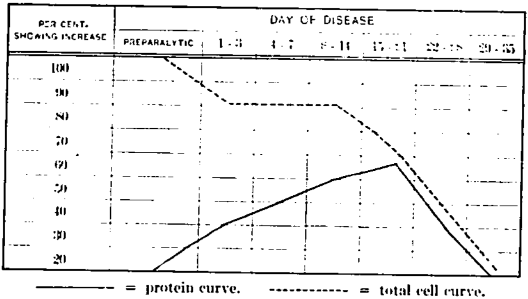
Method of Pandy.—This test was conducted by mixing in a small test-tube 1 c.c. of a saturated aqueous solution of carbolic acid and one drop spinal fluid. The immediate formation of a definite cloud was regarded as a positive reaction.

Method of Gordon.[1]—This test was conducted by placing 1 c.c. of spinal fluid in a small test-tube and adding 0.1 c.c. of 1 per cent. solution of bichloride of mercury in distilled water. The development of a cloud or precipitate after standing an hour was regarded as a positive reaction.

Method of Kaplan.—This test was conducted with 0.1, 0.2, 0.3 and 0.4 c.c. of spinal fluid, but the results expressed in Table IV are based upon the reactions observed with 0.2 c.c. of fluid. This amount of fluid was placed in a small test-tube and 0.3 c.c. of distilled water added, followed by heating until the mixture boiled up twice. Three drops of a 5 per cent. solution of butyric acid in physiological salt solution was added and the mixture carefully underlayed with 0.5 c.c. of a saturated aqueous solution of ammonium sulphate. The reactions were read after standing twenty minutes and regarded as positive if a definite ring was apparent; opalescence only was regarded as negative.

Accordingly we have found the total cell count alone of most value in aiding the diagnosis of acute anterior poliomyelitis; during the earliest stages of the disease the globulin-albumin tests may or may not be positive, and usually are not. In tuberculosis and meningococcic meningitis, on the other hand, the globulin-albumin tests are usually positive in the early stages, due to the greater degree of involvement of the meninges.

Chart 1.—Curves of total cell and protein increase in cerebrospinal fluid of poliomyelitis.



4. Lange's Colloidal Gold Reaction in Acute Anterior Poliomyelitis. While the exact mechanism of the precipitation of colloidal gold and the nature of the substance in cerebrospinal fluid responsible for the phenomenon are unknown it is supposed by many investigators that the precipitating substance is of a protein nature. We have examined the fluids of 38 cases drawn from two to sixteen days after the onset of paralysis with colloidal gold prepared after the method of Miller and his associates,[9] and fulfilling all the requirements of a satisfactory preparation of colloidal gold as emphasized in their paper, to determine if changes occur in poliomyelitis and whether or not these are sufficiently constant to render the test of diagnostic value. The results of these examinations are shown in Chart 2.

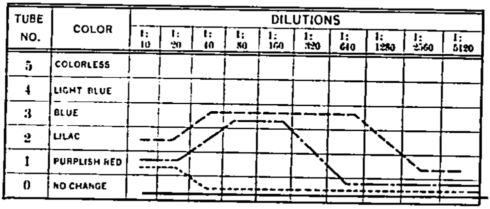
The fluids of 18 cases examined at intervals of two to twelve days after the onset of paralysis showed no color changes at all.

The fluids of 4 cases in the same stages of the disease showed but very slight precipitation in the first two tubes of the series as a 1,100,000,000 reaction. All of these fluids showed an increase of cells and one of them an increase of globulin (Noguchi method).

The fluids of 10 cases examined two to twelve days after the onset of paralysis yielded reactions of the "luetic zone" type with maximum precipitation in the 1 : 40 to 1 : 160 dilutions of fluid with decolorization usually terminating with "blue."

The fluids of 6 cases examined twelve to nineteen days after the onset of paralysis yielded reactions of the "meningitic zone" type characterized by maximum precipitation in dilutions of 1 : 80 to 1:320 or 1:640. All fluids yielded negative Wassermann reactions.

CHART 2.—Summary of results of Lange's colloidal gold reaction with the cerebrospinal fluids of 38 cases of acute anterior poliomyelitis.



———— = observed with the spinal fluids of 18 cases examined 2 to 12 days after the onset of paralysis.

.... = observed with the spinal fluids of 4 cases examined 2 to 12 days after the onset of paralysis.

—.—.—.— = observed with the spinal fluids of 10 cases examined 2 to 12 days after the onset of paralysis ("luetic zone" curve).

— — — — = observed with the spinal fluids of 6 cases examined 12 to 19 days after the onset of paralysis ("meningitic zone" curve).

According to these results, while 40 to 50 per cent. of spinal fluids from cases of epidemic poliomyelitis ranging from the second to the nineteenth day after the onset of paralysis tend to react in a rather constant manner, yielding reactions of the "luetic and meningitic zone" types, similar reactions occur in other forms of meningitis, and we regard the colloidal gold reaction of but helpful diagnostic value; a peculiar or definite curve of precipitation with the spinal fluid of epidemic poliomyelitis did not occur in our experience.

5. MAYERHOFER POTASSIUM PERMANGANATE REDUCTION REACTION IN ACUTE ANTERIOR POLIOMYELITIS. Mayerhofer[10] has proposed the reduction of a decinormal solution of potassium permanganate by 1 c.c. of spinal fluid in an acid medium as an index of the amount of protein substances present and as a method of value in the diagnosis of tuberculous meningitis on the basis that while 1 c.c. of normal spinal fluid usually reduced 2 to 2.3 c.c. of $\frac{N}{10}$ potassium permanganate solution, the fluid of tuberculous meningitis may reduce 3 c.c. or more. Hoffman and Schwartz[11] determined the index on spinal fluids of 3 cases of acute poliomyelitis with readings varying from 2.5 to 3.3 in the acute stage and showing a decline coincident with the disappearance of acute symptoms.

The technic of the test as employed by us is given below.*

We have employed this test in the study of 78 spinal fluids from cases of epidemic poliomyelitis at different stages of the disease ranging from the second to the twenty-first days after the onset of paralysis, with the following reduction indices:

0.7 to 1.1 with the fluids of 6 cases.
1.2 to 1.6 with the fluids of 17 cases.
1.7 to 2.0 with the fluids of 23 cases.
2.4 to 3.6 with the fluids of 31 cases.
6.1 with the fluid of 1 case.

According to Mayerhofer the reduction index may be regarded as increased if over 2.3; the fluids of 32 cases yielded indices over 2.4, indicating that 41 per cent. of the spinal fluids from cases of acute epidemic poliomyelitis in the first three weeks of the infection contain an increased protein content. The determination of the reduction index may be of some practical value in aiding the diagnosis of acute poliomyelitis, as indices of over 2.3 indicate the presence of increased protein due to meningitis.

6. Reduction of Fehling's Solution by Spinal Fluid in Acute Anterior Poliomyelitis. A qualitative Fehling's test† for the presence of dextrose was conducted with 779 spinal fluids from cases of epidemic poliomyelitis in various stages of the disease. Every fluid reduced a portion of the copper solution, but in varying amounts; with 86 fluids the amount of fluid required to effect partial reduction was so large as to indicate that the amount of dextrose was diminished below the normal, but our results in general are in accord with those of previous investigators, indicating that insofar as the qualitative test is concerned there is no constant alteration in the amount of dextrose in the spinal fluid of epidemic poliomyelitis.

7. The Weil-Kafka Hemolysin Reaction in Acute Anterior Poliomyelitis. Normally the blood of over 90 per cent. of persons contains natural antisheep hemolysin, but the cerebrospinal fluid is free of this substance. In meningitis, according to Weil-

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* *Technic of the Mayerhofer Reduction Test.*—Place in a beaker 50 c.c. distilled water, 10 c.c. of diluted sulphuric acid (1 part concentrated sulphuric acid and 3 parts distilled water) and 10 c.c. of a decinormal solution of potassium permanganate; boil slowly for ten minutes and determine the amount of decinormal oxalic acid solution required to reduce the potassium permanganate solution until water clear. In a second beaker (thoroughly cleansed) place 1 c.c. of spinal fluid, 50 c.c. distilled water and 10 c.c. of diluted sulphuric acid. Bring to boiling-point, add 10 c.c. decinormal potassium permanganate solution and boil slowly for ten minutes. Turn off the burner and add slowly the amount of decinormal oxalic acid determined in the previous titration. The contents of the beaker turn brown and gradually become water clear. With a burette add decinormal potassium permanganate until the first appearance of a clear rose color. The amount of $\frac{N}{10}$ permanganate solution required represents the "reduction index" of the spinal fluid.

† *Technic.*—0.5 c.c. each of Fehling's alkaline and copper solutions were diluted with 9 parts of water and 1 c.c. brought to boiling-point in a small test-tube; 1 c.c. of spinal fluid was added followed by brief boiling and the occurrence and degree of reduction noted after standing several minutes.

Kafka,[12] the hyperemia and increased permeability of the vessels and exudative processes results in the passage of this hemolysin into the cerebrospinal fluid. Likewise, hemolytic complement is not found in appreciable amounts in normal cerebrospinal fluid, whereas in acute meningitis this substance may be found. Tests of the spinal fluid for natural antisheep hemolysin and complement have been advocated in the diagnosis of meningitis, and particularly in those types caused by Treponema pallidum.

We have employed this test, with certain modifications,* in the study of a number of spinal fluids from normal persons, and cases of anterior poliomyelitis in various stages of the disease for the hemolysin alone and for the hemolysin and hemolytic complement. The results of these tests are summarized in Table VI.

TABLE VI.—SUMMARY OF RESULTS WITH THE WEIL-KAFKA HEMOLYSIN REACTION IN EPIDEMIC POLIOMYELITIS.

Reaction.	Poliomyelitis fluids.			Control fluids.			Per cent. positive in poliomyelitis.
	Total.	+	−	Total	+	−	
For antisheep hemolysin alone	65	43	22	11	0	11	66
For the hemolysin and complement	43	13	30	4	0	4	30

The spinal fluids of 65 cases examined for antisheep hemolysin alone showed the presence of this substance in 43, or 66 per cent., whereas the fluids of 11 control persons were negative. The majority of the fluids containing the hemolysin were from cases of poliomyelitis in the acute stages of the infection.

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* 1. Technic for ascertaining the presence of complement and antisheep hemolysin in spinal fluid:

In tube No. 1, 5 c.c. of fresh spinal fluid, free from all traces of blood, were mixed with 1 c.c. of a 2.5 per cent. solution of sheep corpuscles (prepared with physiological salt solution).

In tube No. 2 the spinal fluid was replaced by 5 c.c. of physiological salt solution. This tube served as a control.

The tubes were incubated at 37.5° C. for two hours and centrifugated for a few minutes and the reading of the occurrence and the degree of hemolysis immediately taken. Hemolysis in tube No. 1 indicated the presence of the complement and antisheep hemolysin.

2. Technic for ascertaining the presence of natural antisheep hemolysin alone: Five cubic centimeter of spinal fluid free from blood and bacterial contamination and 1 c.c. of a 2.5 per cent. sheep corpuscle solution were mixed in a test-tube. A control in which the spinal fluid was replaced by physiological saline solution was also included. The tubes were incubated at 37.5° C. for one hour. They were then centrifuged and the entire supernatant fluid carefully pipetted off. To the sediment of corpuscles 1 c.c. of hemolysin-free guinea-pig complement diluted 1 to 20 with physiological salt solution was added. The tubes were well shaken, reincubated at 37.5° C. for one hour, centrifuged, and the reading of the occurrence and degree of hemolysis taken at once. The occurrence of hemolysis indicated the presence of natural antisheep hemolysin which sensitized the sheep cells followed by hemolysis after the addition of complement. The natural antisheep hemolysin in guinea-pig complement was removed by adding 0.2 c.c. of packed washed sheep corpuscles to 5 c.c. of clear undiluted complement serum. This mixture was kept in a tumbler of cracked ice for one hour, centrifuged, and the clear complement serum used for the dilution.

The spinal fluids of 43 cases examined for hemolytic complement and the hemolysin showed the presence of these substances in 13, or 30 per cent., of fluids from cases in the acute stage of poliomyelitis. The fluids of 4 control cases were found free of both complement and the hemolysin.

The results indicate that in acute anterior poliomyelitis there is present an increased permeability of the vessels with the passage into the cerebrospinal fluid from the blood of both natural anti-sheep hemolysin and complement and particularly the former; a test for these substances may therefore prove of aid in the diagnosis of this infection.

8. Hauptmann's Saponin Reaction in Acute Anterior Poliomyelitis. Solutions of saponin are capable of hemolyzing the erythrocytes of various animals. Hauptmann[13] has found that the cerebrospinal fluid from persons suffering with various diseases of the central nervous system may contain a substance which inhibits this hemolysis. The inhibiting substance is supposed to be cholesterin and the presence of amounts in the spinal fluid sufficient to inhibit saponin hemolysis is regarded as being due to the presence of degenerating lesions with the liberation of cholesterin from the nervous tissues.

We have tested the inhibiting properties of the spinal fluids[*]

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[*] *Technic of Hauptmann's Saponin Reaction.*—We have modified Hauptmann's original technic and conducted our tests as follows: Nine cubic centimeters of human blood were obtained by venous puncture and mixed with 1 c.c. of a 10 per cent. solution of sodium citrate in physiological salt solution. The blood was then centrifuged and the supernatant fluid withdrawn. The cells were washed three times in physiological salt solution and a 7.5 per cent. suspension in physiological salt solution prepared. The stock solution of saponin was prepared by dissolving 0.5 gram of saponin (Kahlbaum Com.) in 10 c.c. of sterile distilled water. (Solution occurs readily at 37.5° C.). A 1 to 10,000 solution in physiological salt solution was made for each titration from this stock solution. (The stock solution keeps well for one month, after this time the solvent property is greatly delayed.) The titration for dose of saponin was conducted as follows: Into seven test-tubes were placed 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7 c.c. of the *saponin test solution*, 0.8 c.c. *of physiological salt solution*, and 0.5 c.c. of the 7.5 per cent. blood suspension, mixed and incubated at 37.5° C. for one hour. The next dilution of saponin stronger than the one producing complete hemolysis was chosen for the test. The test was then conducted as follows:

Tube 1: 0.8 c.c. clear spinal fluid + determined amount of NaCl + 0.5 c.c. 7.5 per cent. cells.

Tube 2: 0.2 c.c. clear spinal fluid + determined amount of saponin + 0.5 c.c. 7.5 per cent. cells.

Tube 3: 0.4 c.c. clear spinal fluid + determined amount of saponin + 0.5 c.c. 7.5 per cent. cells.

Tube 4: 0.6 c.c. clear spinal fluid + determined amount of saponin + 0.5 c.c. 7.5 per cent. cells.

Tube 5: 0.8 c.c. clear spinal fluid + determined amount of saponin + 0.5 c.c. 7.5 per cent. cells.

Tube 6: 0.8 c.c. NaCl solution + determined amount of saponin + 0.5 c.c. 7.5 per cent. cells.

All tubes were incubated for two hours at 37.5° C. when the reading of the degree of hemolysis was taken, centrifugation being resorted to if necessary.

Tubes No. 1 and 6, being controls, show complete hemolysis; the presence of substances in the spinal fluid inhibiting hemolysis is indicated in the remaining tubes of the series, the degree of inhibition increasing with the larger amounts of spinal fluids.

from 30 cases of acute anterior poliomyelitis during the acute stages of the disease and the fluids of 5 control persons, with negative results in all. Amounts of spinal fluid from the control and poliomyelitis cases ranging from 0.2 to 0.8 c.c. did not produce any appreciable inhibition of hemolysis.

DISCUSSION. The results of these routine examinations and special studies of the spinal fluids from cases of acute poliomyelitis in the recent Philadelphia epidemic have essentially confirmed the findings of Peabody, Draper, Dochez, and other investigators. The greatly desired diagnostic laboratory test for acute anterior poliomyelitis, *and particularly in the early stages and for those cases apt to escape clinical detection*, has not yet been discovered. None of the physical, chemical, or cytological changes found in the spinal fluid are absolutely confined to acute poliomyelitis or characteristic of this disease; while specific antibodies in the serum have been demonstrated by neutralization of the virus *in vitro* as determined by intracerebral injection into monkeys, this is not a practical diagnostic method and attempts to demonstrate complement-fixing antibodies in the serum and spinal fluid have yielded mostly negative results in the experiments of Gay and Lucas[14] and Kolmer and Freese.[15] Likewise, the microparasite of poliomyelitis discovered by Flexner and Noguchi[16] apparently cannot be cultivated from the cerebrospinal fluid and blood during life, but only from the nervous tissues after death by special methods and under exceptional difficulties, and bacteriological examinations therefor are not available for diagnostic purposes. The easily cultivated streptococci and diplococci found in the brain, cord, and spinal fluid of poliomyelitis, and recently given renewed prominence by the reports of Mathers,[17] Rosenow, Towne and Wheeler,[18] and Nuzum and Herzog[19] do not, in our opinion, possess any diagnostic value; the studies of Kolmer, Brown and Freese[20] with these bacteria are in accord with those investigators who have disregarded these microörganisms as the etiological agent of the disease; we regard these cocci as bearing a relation to poliomyelitis similar to streptococci in scarlet fever. Cultures of the cerebrospinal fluid in acute poliomyelitis during life are sterile in many cases and the presence of an easily cultivated bacterium is always suggestive of contamination. . Streptococci are to be found in the tissues of the nervous system and internal organs but only exceptionally in the cerebrospinal fluid during life.

The extent of the changes in the spinal fluid appear to be dependent upon the degree of hyperemia and cellular and serous exudation in the pia mater. As the meningitis produced by the microparasite of poliomyelitis is of an interstitial character rather than a surface infection the alterations of the cerebrospinal fluid from the normal in acute poliomyelitis are not usually as extreme as found in such infections as those produced by the meningococcus and pneumococcus. For this reason the examination of cerebrospinal fluid must be thorough and employ delicate methods for the detection of abnormal changes. Similar changes in the spinal fluid may occur in tuberculous meningitis, and requires very careful and painstaking search for the tubercle bacillus in the differential diagnosis between the sporadic and atypical case of acute poliomyelitis and tuberculous meningitis. Usually no difficulty is encountered in differentiating from such forms of meningitis as those produced by the meningococcus, pneumococcus, and influenza bacillus on the basis of the high total cell counts, presence of polymorphonuclear leukocytes, large amounts of protein, and the specific microörganism in the cerebrospinal fluid in these infections. The spinal fluid in "serous meningitis," or acute meningeal congestion, may resemble the fluid in acute poliomyelitis insofar that the fluid in both conditions may be clear and under pressure; but the absence of protein increase and an increase of cells in the former usually serve in differentiating between the two conditions.

In acute poliomyelitis a total increase of the cells mostly of the small lymphocyte variety, in a clear or occasionally opalescent spinal fluid under increased pressure, appears to be the earliest and most constant change, while an increase of protein with slight or no alteration in the dextrose content are additional data of value, as are probably also the presence of natural antisheep hemolysin and a substance partially precipitating colloidal gold in dilutions of fluid ranging from 1 : 40 to 1 : 320.

SUMMARY. In the recent epidemic of poliomyelitis in Philadelphia the examination of cerebrospinal fluids yielded the following results:

1. The majority of the fluids were water clear or but faintly opalescent when viewed against a black background; but 1 or 2 per cent. of blood-free fluids presented distinct turbidity. Xanthochromia and excessive fibrin formation were not found; about 10 per cent. of the fluids presented a small fibrin coagulum after standing several hours.

2. Seventy-seven per cent. of the fluids from all stages showed an increase of total cells; in 80 per cent. of these the counts were not above 100 cells per cubic millimeter of fluid. An increase of the total cells was found in the preparalytic stage, and this increase was present in 95 per cent. of cases for at least three weeks after the onset of paralysis, after which the number of cells gradually decreased.

3. In over 96 per cent. of fluids from cases after the onset of paralysis the small lymphocyte variety of cells predominated. Polymorphonuclear cells predominated in less than 1 per cent. of fluids, and in over 88 per cent. constituted less than 25 per cent. of the cells present.

4. An increase of protein was found in 32 to 42 per cent. of fluids, and different tests for protein yielded varying results. With the Noguchi test the fluid of 1 of 6 cases in the preparalytic stage yielded a positive reaction; the percentage of positive reactions then became gradually higher to the third week after the onset of paralysis (55 per cent. positive), when a rapid decrease in protein became apparent.

5. During the acute stages of poliomyelitis the fluids of 40 to 50 per cent. of cases yielded a colloidal gold reaction of the luetic and meningitic zone types. All yielded negative Wassermann reactions. The balance of these fluids produced no precipitation at all, or but slight changes in the 1 : 10 and 1 : 20 dilutions.

6. The potassium permanganate reduction test yielded positive reactions of indices over 2.3, with the spinal fluids of 41 per cent. of cases examined from the second to twenty-first days after the onset of paralysis; a high reduction index indicates an increase of protein in the fluid, and may aid, therefore, in the diagnosis of acute poliomyelitis.

7. Every fluid was found to contain sufficient dextrose to reduce Fehling's solution in some degree; partial reduction of the amount of dextrose was suspected with a number of fluids on the basis of a qualitative test.

8. Increased permeability of the meninges was indicated by the presence of natural antisheep hemolysin in the fluids of 66 per cent. of cases in the acute stages and both the hemolysin and a hemolytic complement in 30 per cent. Both of these substances were absent in the fluids of control persons.

9. Substances inhibiting saponin hemolysis were not found in the fluids of cases two to twenty-one days after the onset of paralysis.

10. A definite and absolute diagnostic criterion or laboratory test with the cerebrospinal fluid in acute anterior poliomyelitis has not been discovered. A clear or slightly opalescent fluid flowing under increased pressure, sterile as examined by smear and culture when collected aseptically, poor in fibrin, reducing Fehling's solution and containing an increased number of cells chiefly of the mononuclear variety, are the most constant findings. An increase of protein and a high potassium permanganate reduction index strengthen the diagnosis, while a colloidal gold reaction of the luetic and meningitic zone types and the presence of natural antisheep hemolysin are helpful diagnostic data.

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POST-TRANSFUSION REACTIONS: A REVIEW OF 280 TRANSFUSIONS PERFORMED IN THE WARDS OF PRESBYTERIAN HOSPITAL, NEW YORK CITY.

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Transfusions can now be performed with such ease and safety that their use has become very popular in a great variety of clinical conditions. In fact, the treatment is probably resorted to in many cases in which it does no good, and perhaps does harm to the patient. It is therefore worth while to determine the conditions in which